In-vitro screening for AGE-breaking Activity

The in vitro AGE breaking activity, of the representative compounds of the invention has been studied in the laboratory, by incubating reducing sugar glucose, with protein bovine serum albumin, which resulted in browning of solution and increase in the fluorescence. Fluorescence was used as the criteria to monitor the increased AGE formation.

Example 1A

AGE breaking activity has been confirmed by the screening procedure as mentioned below:

Materials:

Bovine serum albumin (fraction V) (BSA)

Glucose, analytical grade

Phosphate buffered saline (PBS)

Equipment:

Microplate ELISA Reader - Spectramax Plus (Molecular Devices, USA)

Microplate washer, (Bio -Tec Instruments, USA)

pH meter

Methods of experiment: Elisa (Enzyme Linked Immunosorbent Assay)

160 mg/ml of protein, bovine serum albumin, BSA and 1.6M glucose sugar were dissolved in phosphate buffered saline, PBS. Sodium azide was added at 0.02% concentration as a preservative. The solution was filtered asceptically through a 0.22 µM filter and kept for aging at 37°C for 16 weeks. After 16 weeks the solution was dialyzed against PBS, aliquoted and stored at - 20°C.

To determine the AGE breaking activity, 10 µg/ml of the 16 weeks AGE-BSA was incubated with different concentrations of the test compounds at 37°C for 24 hours and AGE breaking activity of the test compounds by ELISA was determined.

ELISA was performed as follows:

1. Different concentrations of 16 weeks AGE-BSA were coated on a microtitre plate as standard. Each concentration is coated in triplicates.

2. The test samples were coated on microtitre plate at a concentration of 5 ng. to 20 ng per well in triplicates.